Research Article

Investigation of the Coagulation Profile in Calves With Neonatal Diarrhoea*

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Abstract: In this study, 20 neonatal diarrhoeic and 10 clinically healthy neonatal calves were used. Venous blood samples were collected from each animal to determine platelet numbers, pH, partial pressure of carbon dioxide (pCO_2), partial pressure of oxygen (pO_2), saturated oxygen (O_2SAT), content of carbon dioxide ($ctCO_2$), actual bicarbonate (HCO_3act), standard bicarbonate (HCO_3std), and actual base excess (actBE) of plasma. Plasma samples were collected from each animal for the measurement of prothrombin time (PT), thromboplastin time (aPTT), thrombin time (TT), and the concentrations of fibrinogen and cross-linked fibrinogen degradation products (D-dimer). Blood pH, HCO_3act, HCO_3std, actBE, $ctCO_2$, and O_2SAT values were significantly ($P < 0.001$) lower in diarrhoeic calves than in healthy controls. PT, aPTT, and TT values were prolonged ($P < 0.005$), and fibrinogen concentration increased ($P < 0.001$) significantly in calves with diarrhoea than in control calves, while D-dimer concentrations were negative in both diarrhoeic and control calves. The results suggest that a compensatory type of disseminated intravascular coagulation (DIC) develops in diarrhoeic calves.

Key Words: Calves, neonatal diarrhoea, coagulation profile

Neonatal İshalli Buzağılarda Koagulasyon Profilinin Araştırılması

Özet: Bu çalışmada 20 neonatal ishalli ve 10 sağlıklı buzağı kullanıldı. Venöz kan örneklerinden trombosit sayısı, kan pH'ı, karbondioksit basıncı (pCO_2), oksijen basıncı (pO_2), oksijen saturasyonu (O_2SAT %), karbon dioksit miktarı ($ctCO_2$), aktüel bikarbonat (HCO_3act) standart bikarbonat (HCO_3std) ve aktüel baz excess (actBE) değerleri ölçüldü. Her hayvandan alınan plazma örneklerinden protrombin zamanı (PT), parsiyel tromboplastin zamanı (aPTT), trombin zamanı (TT), fibrinojen, fibrin yıkımlanma ürünleri (D-Dimer) ölçüldü. Bu çalışmada neonatal ishalli buzağılarda kan pH, HCO_3act, HCO_3std, actBE, $ctCO_2$ ve O_2SAT değerlerinin kontrol grubundan önemli derecede ($P < 0,001$) düşük olduğu saptandı. Neonatal ishalli buzağılarda PT, aPTT ve TT değerlerinde önemli uzama ($P < 0,05$) ve fibrinojen değerinde önemli artış ($P < 0,001$) saptanırken, D-Dimer konsantrasyonları ishalli ve kontrol hayvanlarında negatif bulundu. Çalışmada elde edilen sonuçlar neonatal ishalli buzağılarda kompensatorik tipte dissemine intravaskular kaugulasyonun (DIC) gelişmiş olabileceğini göstermektedir.

Anahtar Sözcükler: Buzağı, neonatal ishal, koagulasyon profili

Introduction

Neonatal diarrhoea, an important disease in newborn calves, causes considerable economic loss in cattle farming. In diarrhoeic calves, endotoxaemia, dehydration, metabolic acidosis, and septic shock are common complications and are associated with death (1,2).

It is known that several Gram-negative bacteria containing lipopolysaccharide (LPS), such as *Escherichia coli* and Salmonella spp., are important causative agents of diarrhoea in calves. Lipopolysaccharide located on cell walls of Gram-negative bacteria play an important role in the development of endotoxaemia. Endotoxin exerts its detrimental effects on the host by interacting with components of the coagulation, inflammation, and immune systems (3,4). During septic shock, the delicate balance of procoagulant, anticoagulant, and fibrinolytic factors that maintain the homeostasis of the coagulation system is disrupted (5).

Endotoxin-induced injury to endothelial cells initiates intrinsic and extrinsic coagulation through exposure of subendothelial collagen and inducing the release of thromboplastin, tissue plasminogen activator, and plasminogen activator inhibitor (6,7). Local or systemic coagulation frequently accompanies sepsis (8-10). Local intravascular and extravascular coagulation events typically occur at sites of inflammation, whereas systemic activation of coagulation results in deposition of microthrombi within microvasculature of multiple organs (11).

With overactivation of the haemostatic mechanism, coagulation factors and thrombocytes are consumed excessively, resulting in thrombin formation in capillary blood vessels. Therefore, ischemia, functional disorders, a tendency for bleeding, and disseminated intravascular coagulation (DIC) may develop in several organs (11-14). The endotoxin-induced cumulative effects are deregulation of the coagulation, inflammatory, immune, and cardiopulmonary systems, and disseminated intravascular coagulopathy, circulatory shock, multiple organ failure, and occasionally death (3,4). Coagulopathies and DIC were diagnosed by measurement of prothrombin time (PT), thromboplastin time (aPTT), thrombin time (TT) (15), concentrations of fibrinogen and crosslinked fibrinogen degradation products (D-Dimer) assay, and platelet counting. D-Dimer assay, in particular, is a newer test for the detection of DIC. D-dimer is a neoantigen formed when thrombin initiates the transition of fibrogen to fibrin and activates factor XIII to crosslink the fibrin formed; this neoantigen is formed as a result of plasmin digestion of crosslinked fibrin (16-18). The D-dimer test is therefore, specific for fibrin degradation products (FDP) (19).

Metabolic profile, blood gas, and acid-base disturbances have been well established in diarrhoeic calves (20-22). However, the coagulation profile has not been well studied and needs to be investigated in diarrhoeic calves. Therefore, the purpose of this study was to investigate the possible alterations in the coagulation profile in neonatal diarrhoeic calves.

Materials and Methods

Ten healthy and 20 neonatal diarrhoeic calves aged between 20 to 30 days and of different breeds raised in the same environment were used in this study. Routine clinical examinations were conducted in all calves.

Blood gas analysis

From each animal, 3 ml of peripheral blood was drawn from the jugular vein into a syringe containing 0.1 ml of heparin. These blood samples were immediately analysed on a blood gas analyser (Chiron Diagnostics, Rapid Lab 248, Cambridge, UK) for pH, partial pressure of carbon dioxide (pCO_2), partial pressure of oxygen (pO_2), saturated oxygen (O_2SAT), content of carbon dioxide ($ctCO_2$), actual bicarbonate of plasma (HCO_3act), standard bicarbonate (HCO_3std), and actual base excess (actBE).

Coagulation tests

To measure PT, aPTT, TT, and fibrinogen concentration 9 ml of blood from each calf was collected in tubes containing 1 ml of 3.8% sodium citrate (Dialab, Paninken, Vienna, Austria). The process was carried out according to the manufacturer instructions with a semiautomatic coagulometer (Diaclot-C1, Dialab). Platelets were counted as described by Coles (23). A D-dimer latex agglutination test (Dimertest latex assay, Dade Behring Marburg GmbH, Germany) was also used to determine cross-linked fibrinogen degradation products (D-dimer).

Statistical analyses

All the values were expressed as the mean and the standard deviations of the mean (mean ± SD). Student's t-test was used to analyse the significance of the differences between the groups as described by Kirkwood (24).

Results

All calves with neonatal diarrhoea had dehydration, enophthalmia, hypothermia, and depression.

Blood gas analysis revealed that pH values were lower in diarrhoeic animals than in healthy calves. HCO_3act, HCO_3std, and actBE values of plasma were lower (P < 0.001) in the diarrhoeic animals than in the control calves. Furthermore, O_2SAT and $ctCO_2$ values were significantly lower (P < 0.001) in diarrhoeic calves compared to measurements obtained in the controls (Table).

Table.   Blood gas values, PT, aPTT, TT, fibrinogen, platelets, and D-dimer in control calves and calves with neonatal diarrhoea. Data are presented as mean ± Sd.

Parameters	Control Group (n = 10) X ± Sd	Diarrhoeic Group (n = 20) X ± Sd
pH	7.44 ± 0.05	7.16 ± 0.03**
pCO_2 (mmHg)	46.94 ± 1.38	46.19 ± 2.64
pO_2 (mmHg)	30.61 ± 0.92	24.72 ± 2.61
O_2 SAT (%)	59.88 ± 1.95	31.25 ± 5.73**
$ctCO_2$ (ml/dl)	32.50 ± 0.83	19.11 ± 1.50**
HCO_3act (mmol/l)	31.70 ± 0.57	17.17 ± 1.39**
HCO_3std (mmol/l)	28.73 ± 0.57	15.34 ± 1.33**
actBE (mmol/l)	6.89 ± 0.79	-10.87 ± 1.87**
PT(s)	17.55 ± 0.62	22.23 ± 1.92*
aPTT(s)	44.81 ± 1.89	48.07 ± 1.92*
TT (s)	19.27 ± 1.07	43.06 ± 4.12**
Fibrinogen (mg/dl)	326.10 ± 18.12	468.57 ± 26.76**
Platelets ($10^3/mm^3$)	320 ± 127	294 ± 118
D-dimer	negative	negative

Significant differences in the values between the diarrhoeic group and control group are indicated by: * $P < 0.05$; ** $P < 0.001$.

In the present study, aPTT, PT, and TT values in diarrhoeic calves were significantly longer ($P < 0.05$) than those in the control group. Moreover, fibrinogen concentrations were higher ($P < 0.001$) in diarrhoeic calves than in healthy calves. There was no significant difference in the number of thrombocytes between diarrhoeic and healthy calves. D-dimer was negative in all diarrhoeic and healthy calves (Table).

Discussion

Neonatal diarrhoea has a high mortality rate and is an important disease of newborn calves and is of major economic importance to cattle farming. Several pathogens, such as viruses, bacteria, and parasites are causative agents of diarrhoea in neonatal calves. Gram-negative bacteria such as Escherichia coli are causative agents, which cause sepsis, endotoxaemia, shock, depression, and diarrhoea in calves (1,2). In this study, all calves with diarrhoea were under 1 month old and they had dehydration, enophthalmia, hypothermia, and depression. These animals were considered to be in septic shock according to the above-mentioned severe clinical signs (2).

Metabolic and haematological alterations also develop in septic events due to the activation of defence mechanisms by Gram-negative bacteria (3,4). Several studies have indicated that leucocytes, thrombocytes, and haemostatic systems were severely affected in septic animals (4,25). In addition to these, elongation in PT, aPTT, and TT increases in fibrinogen degradation products (FDP), and fibrinogen concentrations and thrombocytopenia have been reported in other septic animals (11,26-29).

Blood pH, HCO_3act, $ctCO_2$, and O_2SAT values were significantly lower ($P < 0.001$) in calves with neonatal diarrhoea compared to healthy calves, which indicated the development of metabolic acidosis (20-22,30). Metabolic acidosis observed in this study might have developed due to the reported loss of fluid and electrolytes in diarrhoea (2). Furthermore, metabolic acidosis accompanying inflammation, sepsis, shock, and hypoxia is known to deteriorate the coagulation profile (19). Acidosis has been thought to trigger DIC, most probably via endothelial sloughing, with the attendant activation of factor XII to XIIa and/or XI to XIa and/or platelet release, and activation of the procoagulant system (19,31).

In this study, significant elongations in PT ($P < 0.05$), aPTT ($P < 0.005$), and TT ($P < 0.001$) values were recorded in diarrhoeic calves. The most common causes of prolonged aPTT and PT are reported to be due to liver failure, vitamin K deficiency, and excessive consumption of clotting factors during the development of DIC (26). The prolongation of PT, aPTT, and TT obtained in this study might have resulted from metabolic acidosis (31) and the excessive use of coagulation factors due to the development of DIC (26).

The present study revealed a significant increase in concentration of fibrinogen ($P < 0.001$) in diarrhoeic calves compared to the control calves. The increase in fibrinogen concentration might have occurred due to increased production of acute phase reactant proteins (28), and exceeding the production of fibrinogen on its consumption during the development of compensatory DIC (29).

Furthermore, D-dimer was negative in all control and neonatal diarrhoeic calves, which may be indicative of the possible development of the compensatory type of DIC. On the other hand, D-dimer or fibrin degradation products may not be detectable in the hypercoagulable

state of DIC (11), which may explain the presence of negative D-dimer values in this study. In addition, D-dimer level was regarded as negative in healthy controls according to the test estimation suggested in its procedure.

There was no statistically significant difference in the platelet counts in the diarrhoeic group compared to the control calves. The splenic pool contains 20%-30% of platelets and they can be released into the blood circulating during stress, pain, anxiety, or concurrent disease (5). This may explain and may have contributed to the absence of significant thrombocytopenia in diarrhoeic calves recorded in this study.

In conclusion, blood pH, O_2SAT (%), $ctCO_2$, HCO_3act, HCO_3std, and actBE values were significantly ($P < 0.001$) lower in diarrhoeic calves than in the controls. PT, aPTT, and TT values were significantly prolonged ($P < 0.05$), and concentration of fibrinogen was significantly higher ($P < 0.001$), while D-dimer concentrations were negative in diarrhoeic calves. The results of the present study suggest that the compensatory type of DIC may develop in neonatal calves with diarrhoea. Moreover, these results may also provide important information for managing the treatment, prognosis, and understanding of the pathogenesis of neonatal diarrhoea in calves.

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